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Biofilms in fish processing

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Abstract: The seafood industry is expanding to include traditional wild-caught species, as well as farm-raised species (aquaculture). With this expansion comes different growing and processing technologies. Biofilm formation has been problematic in the aquaculture industry from farm (preharvest) through the processing plant (post harvest). This chapter discusses the three product groups, mollusk, crustacean and finfish, with regard to possible biofilm formation. Of concern is the possibility that the biofilm may contain pathogenic microorganisms, i.e. *Listeria monocytogenes*, *Salmonella* spp., *Vibrio* spp., *Bacillus* spp., *Aeromonas* and *Pseudomonas* spp. These microbes are known to form biofilms and information of their involvement in biofilm formation in the seafood industry is discussed. In the aquaculture industry water is a major source of carrying these microbes from one operation to another. Methods of preventing and cleaning/disinfecting to remove the biofilm, which possibly harbors pathogens, are discussed. Future areas of investigation include the improving of the effluent water system for ponds, filtration/disinfection steps for the recirculating systems and processing plant sanitization.

Key words: biofilm, aquaculture, human pathogens, biofilm removal techniques.

19.1 Introduction

In 2004, an estimated 75% of the world fish production (105.5 million tons) was for human consumption, and the remaining 25% (34.8 million tones) went into non-food production, ie, production of finfish-derived products and oil (FAO, 2006). Fish consumption, which provides a major protein source in the diet, can vary from 1–100 kg/capita depending on the geographical area and individual country. The amount of wild caught fish has become a concern in that natural replacement may not keep up with the demand. Aquaculture, the farming of fish, is helping to meet the demand, and much agricultural land has been converted to aquaculture farming,

particularly in southeast Asia. In 2004, aquaculture contributed an estimated 43% of the available fish consumed, and for some countries this can result in an increase of exported and imported fish. The exporting and importing of fish and fishery products has major trade implications, and the quality and safety of the products can be assessed for detention or rejection in international fish trade.

In the United States, fish is defined in Section 21 of the United States' Code of Federal Register part 123.3 as 'fresh or saltwater finfish, crustaceans, other forms of aquatic animal life (including, but not limited to, alligator, frog, aquatic turtle, jellyfish, sea cucumber, and sea urchin and the roe of such animals) other than birds or mammals, and all mollusks, where such animal life is intended for human consumption' (FDA, 2006). Another term used to describe fish is seafood, which is divided into three categories: finfish (carp, tilapia, catfish), crustacean (shrimp and lobster), and mollusk shellfish (oysters and clams) and will be the category terms used in the discussion of biofilm formation in seafood processing.

In North America, consumption of fish has remained at about 23 kg/capita (1996–2006). A report issued by United States General Accounting Office (GAO, 2004) on food safety related to the seafood program in 2004, stated that 80% of the consumed seafood was imported and that eating contaminated seafood (crustaceans and finfish) resulted in about 15% of the reported food borne outbreaks in the US, which 'is a greater percentage than either meat or poultry even though meat and poultry are consumed at 8 and 6 times the rate of seafood, respectively.' Bacterial pathogens were listed as the cause of the seafood-related illnesses (GAO, 2004). The United States Food and Drug Administration collected and tested 11 312 imported and 768 domestic seafood samples from 1990 to 1998 and reported that overall 7.2% of imported and 1.3% of domestic seafood were positive for *Salmonella*. Similar survey results for seafood products were reported from other countries: Norway (Guerin *et al.*, 2004), Italy (Ripabelli, 2004); Spain (Herrera *et al.*, 2006); Austria (Suppin and Smulders, 2005); and Ghana (Ampofo and Clerk, 2002). These survey results indicated an increased awareness of the role of aquaculture in the spread of pathogens. In a review of food borne microbial pathogens of aquaculture-grown seafood, Greenlees *et al.* (1998) reported on the food borne microbial pathogens associated with these products and identified *Escherichia coli* and *Salmonella* as bacterial inhabitants of pond water. S*almonella* was isolated from both fish and shellfish, whereas *E. coli* was isolated only from finfish. Andrews *et al.* (1977) surveyed retail fresh and frozen channel catfish (*Ictalurus punctatus*) and reported that the number of samples positive for *Salmonella* in farm-raised catfish varied with the seasons with a 0.9% incidence for January to March versus 5.7% for July to September. Huss *et al.* (2000) reviewed the hazards of consuming seafood and identified both *Salmonella* and *E. coli* O157:H7 as pathogens contaminating seafood. Atanassova *et al.* (2008) surveyed sushi and reported the isolation of both

Salmonella and *E. coli* from fresh fish with the prevalence of *E. coli* higher than *Salmonella*. As more reports become available, there will be an increased emphasis on food safety of seafood products.

19.2 The water environment

With the awareness that seafood products can become contaminated with both human pathogens and spoilage bacteria, the attachment and survival of these microbes are now being studied and reported. The water environment is not particularly rich in nutrients. With a biological oxygen demand of <2 mg/liter, water has a nutrient content sufficient for growth and survival of microorganisms such as *Escherichia coli* O157:H7 (Rajkowski and Rice, 1999), *Salmonella* spp., and *Vibrio cholerae* (Rajkowski *et al.*, 1996), *Pseudomonas* and *Shigella* (Rajkowski and Rice, 2001), and *Aeromonas* (Palumbo *et al.*, 1996). Biofilms developing from estuary or marine water sources were documented by Zobell (1943) over 60 years ago. Bacterial biofilms colonize the tissue of fish, including mollusks and crustaceans and can reflect the health of the animal (Geesey *et al.*, 1992). One area of study is how the bacteria from the nutrient-limited water environment attach and form biofilms. Kjelleberg and Hermansson (1984) studied the attachment of *Vibrio* and *Pseudomonas* during starvation and concluded that there were marked changes in the cells physiochemically. The cells irreversibly bound to the solid surfaces and remained stable during starvation. With microscopic examination, it was reported that the cell surface became rough, which seemed to aid in attachment. Environmental stress, including changes in temperature and pH, was reported to affect the ability of *Listeria monocytogenes* Scott A to attach to food contact surfaces when grown in phosphate buffer, a nutrient deprived medium (Smoot and Pierson, 1998a; 1998b).

19.3 Microorganisms of concern in biofilm formation

Biofilms on fish surfaces and bacteria from marine water source can contaminate seafood processing facilities. *Pseudomonas*, *Vibrio*, *Staphylococcus*, *Bacillus*, and *Aeromonas* are among the genera that can cause food borne illness and form biofilms (Cahill, 1990). Other genera that were isolated from seafood included *Listeria*, *Salmonella*, and *Shigella* biofilm formers (Somers *et al.*, 1994).

Listeria monocytogenes

Listeria monocytogenes is considered a pathogen of seafood. In flowing systems Sashara and Zottola (1993) demonstrated that *Listeria* attaches and forms biofilms. This microbe is water borne and was isolated from fresh

channel catfish, *Ictalurus punctatus* (Erdenlig *et al.*, 2000), from fresh water fish in India (Jallewar *et al.*, 2007), and was reported to survive and grow on refrigerated fish fillets (Jørgensen and Huss, 1998; Augustin *et al.*, 2005). *L. monocytogenes* was identified as a contaminant of crab meat (Brackett and Beuchat, 1990) and in vacuum-packaged cold-smoked salmon fillet (Heinitz *et al.*, 2000). The control of *L. monocytogenes* biofilms is covered in Chapter 7.

Salmonella *spp.*

Salmonella spp. were identified as contaminants of farm raised shrimp (Zhao *et al.*, 2006). This water borne contaminant can develop as a biofilm by attaching to the crustacean's chitin during growth and can be internalized during feeding. More recently, *Salmonella* was identified as the cause of an outbreak following the consumption of smoked eel in Germany (Fell *et al.*, 2000; Heinitz *et al.*, 2000). *Salmonella* was isolated from fresh fish in Iran (Basti *et al.*, 2006). Shin *et al.* (2004) reported that the use of antimicrobial ice containing chlorine dioxide effectively lowered the levels of *Salmonella* and *L. monocytogenes* on the surface of fish.

Vibrio *spp.*

Vibrio spp. are recognized as water borne pathogens. In the estuary environment, vibrios are associated with pollution (Miller *et al.*, 2006). When polluted estuaries are found, these areas are closed for harvesting fish, particularly mollusks and wild caught finfish (Chen *et al.*, 2006). Removal or reduction of vibrios in the marine environment is temperature dependent, and the bacteria are found in higher levels when the water temperature ranges from 14 to 19°C (Kaneko and Colwell, 1973). Harvesting is not recommended during those times. Biofilms containing vibrios can occur on the zooplankton in fresh water (Kaneko and Colwell, 1975). When the zooplankton are ingested by mollusks, which are filter feeders, so is the pathogen. Mollusk can accumulate this pathogen internally and on the shell surface as biofilms. Removal from the surface can be done through a brushing process; however, the internalized bacteria are more difficult to remove. Chilling immediately after harvesting is one procedure, and placing the mollusks in sterile water for a set time for reduction in the number of vibrios is another. High pressure processing has been suggested as a means to destroy the pathogen in internalized biofilms (Lopez-Cabellero *et al.*, 2000; He *et al.*, 2002). More recently vibrios were identified as bacterial contaminants on finfish, which can occur when the fish are raised in contaminated water or through cross contamination during the filleting process (Fouz *et al.*, 2006).

Vibrio parahaemolyticus is recognized as a human pathogen of concern and is associated with seafood borne illness. This microbe has been isolated from estuary water in Oregon and Washington (Chiu *et al.*, 2007), Texas and New York (DePaola *et al.*, 2000), China (Yano *et al.*, 2006), Brazil

(Riberiro, 2006), northern Mexico (Gomez-Gil, 2007), and Southern Thailand (Vuddhakul *et al.*, 2006). This pathogen is mainly associated with contaminated mollusks, which are eaten raw but has also been isolated from other seafood products, such as shrimp and finfish. Dawson *et al.* (1981) suggested that adhesion (biofilm formation) is a survival strategy of marine vibrios during starvation (nutrient limiting environments). In his review of *V. parahaemolyticus*, Levin (2006) describes the genes responsible for the formation of the lateral flagella, which results in swarmer cells when grown on solid media – the first step in biofilm formation. Kim and McCarter (2007) studied the gene that controlled the expression of lateral flagella and reported that ScrG, a GGDEF-EAL protein, regulated swarming and attachment of *V. parahaemolyticus* (biofilm formation). Shime-Hattori *et al.* (2006) reported that two type IV pili are involved in the formation of biofilms as a strategy for survival of *V parahaemolyticus* in the environment.

Vibrio vulnificus is now recognized as an emerging fish pathogen in the aquaculture environment, as well as an important human pathogen. In the aquaculture of both eel and tilapia, this microbe causes infection of the fish and results in economic loss (Fouz *et al.*, 2006).

Bacillus *spp.*

Bacillus spp. can become part of biofilms either as bacterial spores or as vegetative cells. However, since most biofilms form during the starvation state, most likely the *Bacillus* cells are in the spore form. Rahmati and Labbe (2008) examined fresh and processed retail seafood samples for the presence of *Bacillus cereus*. The pathogen was isolated from seafood samples at levels ranging from 3.6 to greater than 1000 CFU/g. The strains possessed both the hemolysin BL and nonhemolytic enterotoxin or only one of two toxins. Therefore, seafood is a vehicle for food borne illness caused by *B. cereus*.

Aeromonas

Aeromonas. Within the estuary and aquaculture environments, *Aeromonas hydrophila* was identified as a finfish pathogen and as a possible human pathogen. This microbe infects the finfish resulting in economic loss. If the finfish contaminated with this pathogen are harvested and improperly handled or prepared, *Aeromonas* can grow during refrigerate storage (Leung *et al.*, 1992; Fapohunda *et al.*, 1994; Fernandes *et al.*, 1998), and this may result in food borne illness.

Pseudomonas *spp.*

Pseudomonas spp. are opportunistic pathogens and are the major seafood spoilage organisms. They are slimy biofilm formers and histamine producers causing both quality and safety issues (Geesey *et al.*, 1992). *Pseudomonas*

species were the dominant bacteria on fish skin regardless of time of the year (Tryfinopoulou *et al.*, 2002).

Other bacteria
Other bacteria: *Escherichia coli* and *Shigella* spp. were isolated from seafood (Greenlees *et al.*, 1998; King *et al.*, 2004) and have been shown to be associated with biofilms.

19.4 Control or removal of biofilms during seafood processing

19.4.1 Preharvest

Open air ponds
Control of bacteria in the pond environment is difficult. Figure 19.1 shows an aerial view of a catfish farm in Stoneville, MS. The pond water is fed and drained from a natural source, and the depth of the pond varies depending on the catfish growth. Since the bacteria in ground water can attach and develop biofilms on the indigenous zooplankton, removal is almost impossible. In geographic areas where human pathogens cannot be removed from the effluent, the ponds are drained, lined with heavy plastic, and the water disinfected before flowing into the pond. When ground water is chlorinated with a residual chlorine content of >0.1 mg/l, no *L. monocytogenes* or *Salmonella* were recovered (Kampelmacher and van Noorle Jansen, 1974). These plastic lined ponds can now be cleaned with similar protocols using Good Aquaculture Practices (Koonse, 2005) in order to reduce bacterial attachment to the zooplankton and thus also on the finfish or shrimp.

During harvesting, baskets or buckets are used to move the seafood from the pond to the transporting vehicle, and these can be made of either metal

Fig. 19.1 Aerial view of catfish farm in Stoneville, Mississippi. Pond water is fed through a natural source. The pond depth varies depending on the size of the catfish.

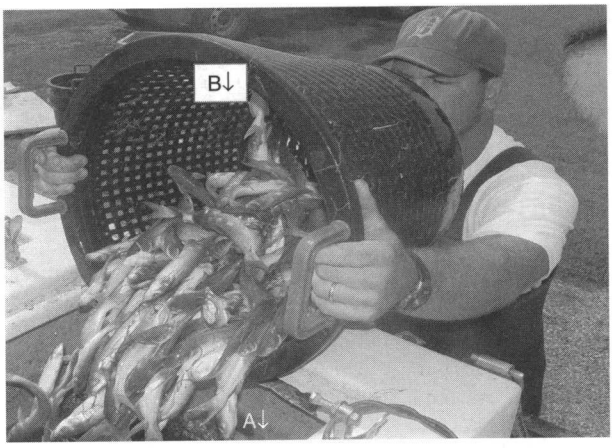

Fig. 19.2 Harvesting of catfish using plastic basket. The arrow at 'A' indicates biofilm formation in transportation tank at the water air interface. The arrow at 'B' indicates spaces where water plants and dirt collect.

or plastic. This equipment, shown in Fig. 19.2, can have trapped particulate matter and floating vegetation, and bacteria can attach onto the basket or bucket with possible biofilm formation. Regarding transport of seafood, vehicles should be sanitized between uses with potable water. During transport, the fish are tightly packed and contamination on the surface of the fish transfers to the surfaces of the transport vehicle. Good Aquaculture Practices are needed to prevent the spread of bacteria.

Recirculating systems

Another area of pre-harvest seafood processing in which biofilms containing human pathogens and spoilage microbes can occur is in recirculating aquaculture system tanks shown in Fig. 19.3. Aquaculture farming in these tanks can be done using either fresh or salt water. Biofilm formation can occur on the surfaces of all materials used: buna-N rubber, polyvinyl chloride (PVC), chlorinated PVC, glass, fiberglass, and stainless steel. Notice in Fig. 19.3, the biofilm on the tank surface in contact with the water, which is a reservoir for bacterial recontamination. Water quality is a major concern, and it is recirculated through a filtration and disfection system (Fig. 19.4 – catch tank of system) to remove uneaten food and the animal waste. Notice the biofilms formed on the pipe water-air interface indicated in the photograph. In some systems the water is passed through a sand filtration apparatus (Fig. 19.5) to remove particulate matter, but the bacteria are not always trapped in the sand filter and further disinfection steps are applied. The most commonly used intervention is ultraviolet light (UV). When the bacteria attached to particulate matter (excess food particles or

Fig. 19.3 Recirculating tank. A) Filter where the water is removed from the recirculating tank, arrow indicates where biofilms form on the filter; B) Air water interface, arrow indicates where biofilms form on tank surface.

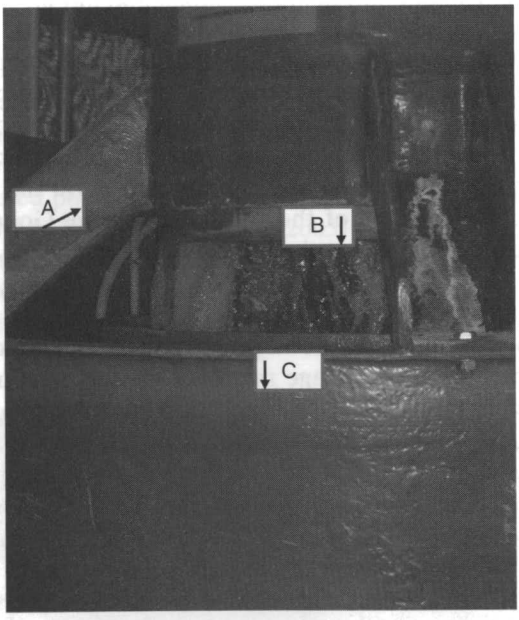

Fig. 19.4 Water exiting the sand filtration system. A) Water pumped to top of sand filter; B) Water exiting sand filter; C) Water overflowing the catch tank for reintroduction into recirculation fish tank.

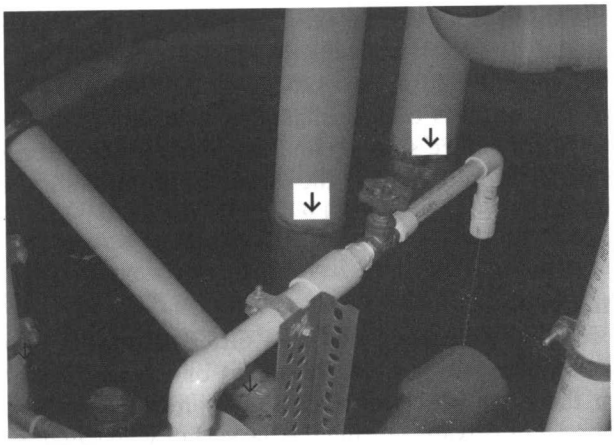

Fig. 19.5 Water holding tank of filtered water before re-entry into growth tank. Arrows indicate places where biofilms form.

fish waste) and are not removed by filtration, UV light was not 100% effective to inactivate the bacteria (Sharrer *et al.*, 2005). The effectiveness of dissolved ozone on water borne microflora has been examined (Bullock *et al.*, 1997; Summerfelt *et al.*, 1997), and there is concern that the residual dissolved ozone would be toxic to the finfish. Summerfelt *et al.* (2004) showed that dissolved ozone can be inactivated by using UV light. Further studies showed that using ozone followed by UV light was an effective process to inactive the bacteria (Summerfelt, 2003; Sharrer and Summerfelt, 2007).

Pipes are used in recirculation systems. The flow characteristics of the liquid in these pipes influence biofilm development. Stoodley *et al.* (1999) studied biofilm formation under laminar and turbulent flow and after increasing the liquid's nutrient content. They reported that the structure of the biofilms was different based on the type of flow. Under laminar flow, the biofilms were patchy and consisted of circular cell clusters, whereas under turbulent flow, the biofilms were patchy and consisted of ripples and streamer type formation. When the nutrient content of the fluid was increased under both laminar and turbulent flow, the cell cluster grew rapidly (laminar) and the biofilm thickened and the ripples disappeared (turbulent).

In addition to the water flow characteristics, the pipe material affects biofilm formation. Kerr *et al.* (1999) reported that medium density polyethylene and unplasticised polyvinyl chloride supported the lowest numbers of bacteria in a steady state biofilm. Further research is needed in this area to determine which piping material would inhibit the formation of biofilms.

After the seafood is harvested, the recirculating system is cleaned and sanitized. King *et al.* (2004) showed that proper cleaning and sanitizing with sodium hypochlorite or peroxacetic acid is effective in removing biofilms from the surfaces of materials. Proper cleaning/sanitizing of the recirculating system before start-up and maintaining the filtering/disinfection of the water would reduce the bacterial populations involved in biofilm formation.

19.4.2 Postharvest

Postharvest seafood processing methods differ somewhat depending on where the seafood is grown, i.e. wild caught or aquaculture grown. Wild catches from the open seas are processed by more traditional methods, either commercial or private. During the harvest of wild catches, preventing bacterial growth and biofilm formation on processing or cutting surfaces and knives for any of the above mentioned pathogens is the first step. For wild catches, the finfish are deheaded and the guts removed. The ship's deck then becomes contaminated with particulate matter, and bacteria attach to surfaces. The seafood is iced during transport. Control of bacterial growth is temperature dependent, requiring a quick decrease in temperature immediately after harvesting and continuance the cool temperature during transport (Bao *et al.*, 2007). Proper cleaning and sanitizing of the harvest vessel and equipment used is essential to prevent biofilm formation and spread of these pathogens.

Aquaculture harvesting processes entail removal of fish from ponds and placing the fish into transportation vehicles. The fish arrive alive at the processing plant. Depending on the variety, finfish are processed by descaling and/or deskinning and/or deboning. The end product can be fillets or cut portions either shipped unfrozen on ice or frozen. Shrimp are collected, placed on ice, and brought to processing plants. The shrimp may then be deheaded, shelled, deveined, or cooked. If the heads are not removed, the shrimp may be soaked in an antioxidant/preservative agent to reduce shell oxidation.

19.4.3 Mollusk processing

Biofilms can be found attached to the surface of the mollusk shell and can be removed by proper cleaning. However, if not removed, the biofilms can become a source of cross contamination, particularly of the ice used for cooling. Mollusks are filter feeders, and the bacteria can accumulate within the flesh (Miller *et al.*, 2006). Physical removal of bacteria from the flesh would cause a quality issue. The US Food and Drug Administration is now recommending that upon harvest, oysters should be refrigerated immediately to prevent the growth of *V. parahaemolyticus*, a major problem with raw oysters. Such chilling can also inactivate other temperature-sensitive

vibrios. Another intervention step involves placing the mollusk in clean water for a period of time to dilute out any pathogenic microbes, but this step would also require recirculation of the water and filtration and disinfection processing steps.

Some mollusks are sold un-cooked after the meat is removed from the shell and packaged. Some packaged mollusks are given a mild heat treatment, which inactivates the microbial population, but does not remove the biofilm. Other means of inactivating microbes from the raw mollusks are gamma irradiation, which was approved for use on mollusks in the United States (FDA, 2005) and high pressure treatment (Lopez-Cabellero *et al.*, 2000; He *et al.*, 2002). In areas where harvesting occurred from known polluted estuaries or aquaculture environments, it is recommended that mollusks be cooked before eating.

19.4.4 Crustacean processing

Aquaculture-raised shrimp have been found to be contaminated with *Salmonella* spp., *L. monocyogenes*, *Vibrio* spp., and *Shigella* spp. During shrimp processing, under conditions where freezing or reduced storage temperatures are used, the vibrios become less problematic. However, other microorganisms were reported to survive the freeze/thaw cycle (Rajkowski, 2007). All of the microorganisms are biofilm formers and survive in the water environment. As biofilms, they are known to attach to the crustacean chitin (skin) and form biofilms, as well as becoming internalized since the crustaceans are also filter feeders. Physical removal of bacteria from the outer skin would cause quality deterioration. During processing, crustaceans are deheaded, deveined (gut removed) and individually quick frozen (considered raw) or cooked with or without the outer skin. If cooked, a glaze usually containing a polyphosphate is applied. Some researchers consider this chemical application as an antimicrobial step, but polyphosphates are also water binding agents and can reduce drip when the product is thawed, reducing the possibility of cross contamination. It is recommended that these frozen products be cooked or blanched before eating.

19.4.5 Seafood finishing processes

Seafood arrives at the processing plant with biofilm on their outer skin, which may transfer to equipment during processing. Trisodium phosphate was shown to inactivate attached *Salmonella* and *L. monocytogenes* on catfish; however, the skin mucus slightly decreased the antimicrobial effect (Kim and Marshall, 2002). Some finfish varieties lend themselves to further processing by heat, i.e. canning of salmon and tuna. Although this thermal processing step does not physically remove biofilms, the bacteria are inactivated.

Canned salted anchovies and sardines are traditional foods in the Mediterranean area. During harvesting, the biofilms that formed in the fish boxes were reported to contain *Salmonella* spp. and *Staphylococcus aureus*, and cross contamination of the fish occurred. In order to control these pathogens, a salt ripening period of 90 days was shown to provide a safe fish product (Arkoudelos *et al.*, 2003). Another process that is used particularly for salmon is cold or hot smoking (Heinitz and Johnson 1998). *Listeria monocytogenes* is a problem in these smoked packaged products, and the packaging process was identified as an area in the plant where contamination for the hot or cold smoked product occurs due to biofilm formation on the fresh salmon. Controls for this pathogen during processing are similar to other food plants. HACCP requires thorough cleaning and sanitizing in the finfish industry (Lupin, 2003).

19.4.6 Removal of biofilms at the processing plant

The equipment and materials used for fish processing are similar to those used in other food plants and include buna-N rubber, polyvinyl chloride (PVC), chlorinated PVC, glass, fiberglass and stainless steel (Ronner and Wong, 1993; Kerr *et al.*, 1999; Joseph *et al.*, 2001). Biofilms were shown to form on these materials, and various sanitizers were tested. Sodium hypochlorite and peracetic acid containing sanitizers were found to be more effective than quaternary ammonium or ozone in the reduction of microflora from processing materials where seafood biofilms developed (King *et al.*, 2004). Bagge-Ravn *et al.* (2003) reported that *Pseudomonas* spp., were the dominant microflora remaining following cleaning and disinfection. *Pseudomonas* is a spoilage organism in seafood. It is recommended that a rinse type cleaning plan be replaced by a more vigorous method followed by use of an appropriate sanitizer. Sharma *et al.* (2005) showed that vigorous methods applying microbubbles to the cleaning liquid flow can stimulate microbial detachment from surfaces. For biofilm inactivation, a residual chlorine level was shown to be the most effective sanitizer (LeChevallier *et al.*, 1988).

19.5 Prevention

In his review of potential hazards in aquaculture fish, Lupin (2003) proposes strategies to reduce the risk, which includes identification of the critical control points in aquaculture. Proper aquaculture techniques using good practices for farmers as outlined by the FDA have shown that Good Aquaculture Practices are essential. Good water quality is the first step in prevention. The water should be disinfected before use in ponds and in recirculating systems. A HACCP program in any processing plant would require proper clean-up of particulate matter, washing, and sanitizing. Plant personnel

should be trained to give them an understanding that removal of adhering microflora is essential for Good Hygienic Practices.

19.5.1 Biofilm inactivation

Various reports have been published describing methods to inhibit the growth of microorganism on raw seafood. Gamma irradiation is proposed for finfish and inactivates pathogens at low levels of irradiation. Rajkowski (2008) reported that seafood isolates had irradiation destruction values similar to *L. monocytogenes* strains isolated from other sources. Doke (1990) reported that low dose irradiation was effective for disinfecting sun-dried fish. Shelf life extension was achieved by pretreatment with ozone (Gelman *et al.*, 2005) or nisin/lactoperoxidase (Elotmani and Assobhei, 2003; Neetoo and Chen, 2008) before cold storage. Ultraviolet light was also used to reduce pathogens on raw finfish fillets giving about a one log reduction (Ozer and Demirci, 2006) and increased the shelf life of iced vacuum packaged fish by 4 days (Huang and Toledo, 1982). UV-light was shown to be an effective water and smooth surface disinfectant (Liltved and Landfald, 2000).

19.5.2 Research needs

Aquaculture farming is a relatively new practice. Much is still unknown about the process and where biofilm formation can become problematic. Research is needed to improve the quality of the effluent water system for ponds, filtration/disinfection steps for the recirculating systems, and the processing plant.

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